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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07C 237/04, A61K 31/16, C07D 295/14, A61K 31/445 — A1

(11) International Publication Number: WO 98/25884

(43) International Publication Date: 18 June 1998 (18.06.98)

(21) International Application Number: PCT/GB97/03444

(22) International Filing Date: 15 December 1997 (15.12.97)

(30) Priority Data:

9625942.9	13 December 1996 (13.12.96)	GB
9714100.6	3 July 1997 (03.07.97)	GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FURTHER ANTHRAQUINONES WITH BIOLOGICAL ACTIVITY

(57) Abstract

Novel substituted anthracene–9,10–diones and their use in the inhibition of telomerase activity and/or in the treatment of cancer.

FURTHER ANTHRAQUINONES WITH BIOLOGICAL ACTIVITY

The present invention relates to anthraquinone compounds, processes for their production and their use as inhibitors of telomerase.

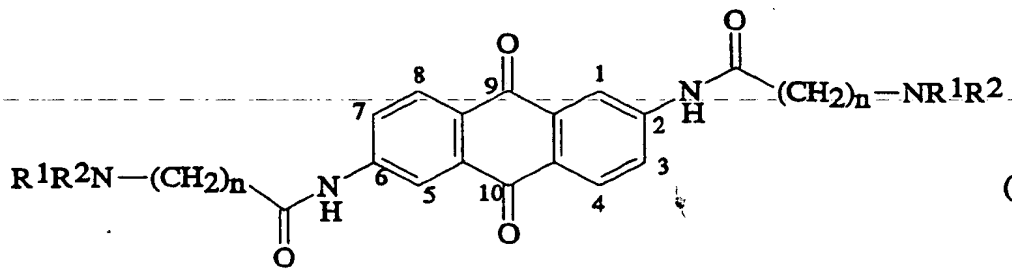
Eukaryotic cells contain chromosomes which divide and replicate during cell division. The ends of the chromosomes - telomeres - comprise tandem repeats of simple DNA sequences. These telomeric repeat sequences are essential for replication although in most normal cell types the length of the telomere is shortened by the process of replication. Cell senescence is closely correlated with a progressive reduction in the number of these repeats, and it is believed that senescence may be caused by a failure to maintain the length of the telomeres.

Further evidence for this can be found in the fact that germ cells and immortalized cancer cells do not suffer the same reduction in the length of telomeres during cell division, due to the activity in these cells of the telomerase enzyme. This enzyme is a ribonuclear protein containing an RNA template for the synthesis of the tandem repeat units of the telomeres.

Almost all tumor cells have shortened telomeres, which are maintained at constant length and are associated with chromosome instability and cell immortalization. The enzyme telomerase adds the telomeric repeat sequences onto telomere ends, ensuring the net maintenance of telomere length in tumor cells resulting in successive rounds of cell division (D. Sun *et al*, J.Med. Chem., 40:2113-2116 (1997)).

Telomerase activity can be found in about 85 to 90% of human tumour cell types, including leukaemias, small cell and non-small cell lung cancer, myeloma, lymphoma, prostate, colon, head and neck, melanoma, Hepatocellular carcinoma, bladder, ovarian, breast and gastric cancers.

WO91/00265 (Neidle *et al*) discloses anti-cancer agents which are anthraquinones of formula (1):

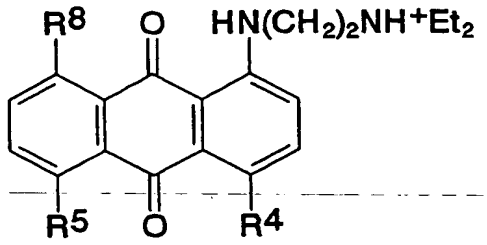


(1)

in which n is 1, 2 or 3; and R^1 and R^2 are each independently an ethyl, hydroxyethyl or hydroxymethyl group; or R^1 and R^2, together with the nitrogen atom to which they are attached, form a cyclic group which is a 1-piperidino, 2- or 4-(2-hydroxyethyl)-1-piperidino, 2-hydroxymethyl-1-piperidino, 4-(2-hydroxyethyl)- or 4-methyl-1-piperazino, or 4-morpholino group; or a pharmaceutically acceptable salt thereof.

Agbandje *et al*, J. Med. Chem., 35: 1418-1429 (1992) describes 9,10-anthraquinones which are examples of the compounds of formula (1) above and allegedly have potential as anticancer agents.

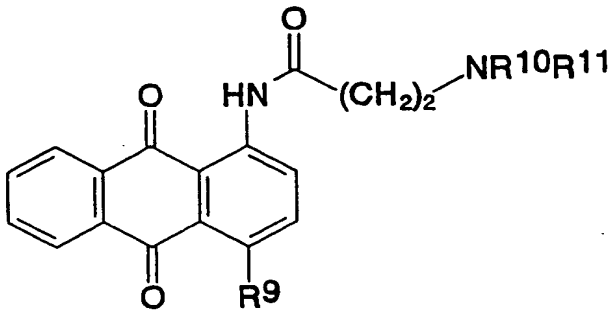
Tanious *et al*, Biochem., 31: 11632-11640 (1992) describes DNA-binding agents which are examples of the 9,10-anthraquinones of formula (1) above and four 9,10-anthraquinones of formula (2):



(2)

in which firstly R^4, R^5 and R^8 are all hydrogen, or in the other three compounds one of R^4, R^5 and R^8 is $NH(CH_2)_2NH^+Et_2$ while the other two of R^4, R^5 and R^8 are hydrogen.

Collier and Neidle, J. Med. Chem., 31: 847-857 (1988) describes a series of 1- and 1,4-substituted amidoanthraquinones of formula (3) that bind to DNA (and thus can be cytotoxic).



(3)

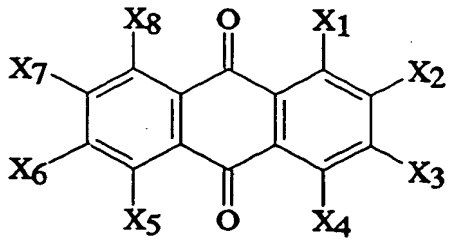
in which R^{10} and R^{11} are each independently an ethyl group; or R^{10} and R^{11} together with the nitrogen atom to which they are attached represent a heterocyclic group which is a 1-piperidino, 4-hydroxypropyl-1-piperazino or 2-hydroxyethyl-1-piperidino group; R^9 is hydrogen or $NHCO(CH_2)_2NR^{10}R^{11}$, in which R^{10} and R^{11} are as defined above.

Some of the compounds of formulae (1), (2) and (3) above have been proposed as anti-cancer agents although to date none have been developed beyond *in vitro* studies because they have been found to have only moderate

.activity in conventional *in vivo* tumour cell lines, and moderate activity against animal models for cancer (Agbandje, M. PhD thesis, University of London, 1989).

However we have investigated compounds within the scope of formulae (1) and (3) above and surprisingly found that these compounds are inhibitors of telomerase. These findings have enabled us to develop novel compounds which also have this activity. The anthraquinones of formula I and II of the present invention have extended planar aromatic groups suitable for intercalation, together with at least one side-chain, each having a planar group at one end such as an amide which is itself attached to the aromatic chromophore, together with a neutral amine or cationic group at the other end. The compounds of the present invention preferably have two side-chains.

Thus in a first aspect the present invention provides novel anthraquinones of the formula I and pharmaceutically acceptable acid addition salts and quaternary ammonium salts thereof:



(I)

in which:

each of X_1, X_4, X_5 and X_8, which are the same or different, is H, $HNCO(CH_2)_nNR^1R^2$, OH, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of X_1, X_4, X_5 and X_8 is $HNCO(CH_2)_nNR^1R^2$, and at most three of X_1, X_4, X_5 and X_8 are $HNCO(CH_2)_nNR^1R^2$, and provided that when X_1 and X_4 are both $HNCO(CH_2)NR^1R^2$ X_5 or X_8 is $HNCO(CH_2)_nR^1R^2$;

each of R^1 and R^2, which are the same or different, is an unsubstituted or substituted alkyl group or R^1 and R^2 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of X_2, X_3, X_6 and X_7, which are the same or different, is H, an unsubstituted or substituted alkyl group or halogen;

provided that:

when X_1 is $HNCO(CH_2)_nNR^1R^2$, each of X_2 to X_8 is hydrogen and n is 2, either R^1 and R^2 do not both represent ethyl, or R^1 and R^2 together with the nitrogen atom to which they are attached do not represent piperidino or 2-hydroxymethyl-piperidino.

Preferably, when more than one of X_1, X_4, X_5 and X_8 is $HNCO(CH_2)_nNR^1R^2$ each group R^1 is the same and each group R^2 is the same.

Preferably, the anthraquinones of formula I contain two $HNCO(CH_2)_nNR^1R^2$ groups. More preferably, X_1 and X_5 or X_1 and X_8 are $HNCO(CH_2)_nNR^1R^2$. Still more preferably, each of X_2, X_3, X_4, X_6, X_7 and X_8 is hydrogen and X_1 and X_5 are $HNCO(CH_2)_nNR^1R^2$ or each of X_2, X_3, X_4, X_5, X_6 and X_7 is hydrogen and X_1 and X_8 are $HNCO(CH_2)_nNR^1R^2$. Preferably R^1 and R^2 are methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. More preferably R^1 and R^2 are the same or R^1 and R^2 together with the nitrogen atom to which they are attached form a heterocyclic group. Preferably the heterocyclic group is a 4 to 8 membered ring, for example a hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or 1-piperidino group which is unsubstituted or substituted with at least one C_1-C_6 alkyl group and/or at least one hydroxy group. More preferably, the heterocyclic group is an unsubstituted hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group or a 2-

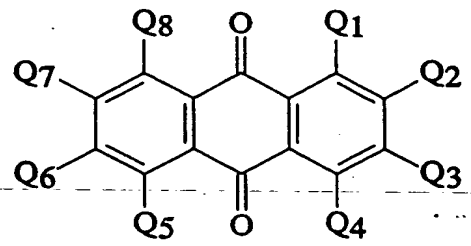
hydroxymethyl-piperidino group. The heterocyclic group may be a bicyclic ring such as an azabicyclo octano ring, for example 1,3,3-trimethyl-6-azabicyclo[3.2.1]octano. Preferably n is an integer of from 1 to 4, for example 1, 2 or 3, most preferably 2.

If R^1 and R^2 are not the same, preferably at least one of R^1 and R^2 is hydrogen or C_1 to C_6 alkyl. Most preferably at least one of R^1 and R^2 is hydrogen, methyl or ethyl. For example, R^1 is 2-hydroxyethyl and R^2 is ethyl, R^1 is methyl and R^2 is hydrogen, R^1 is $CH_2CH_2N(C_2H_5)_2$ and R^2 is methyl or R^1 is $CH_2CH_2NHCH_3$ and R^2 is methyl.

A substituted or unsubstituted alkyl group typically contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH, halogen, NH_2, $N(C_1\text{-}C_6$ alkyl)H and $N(C_1\text{-}C_6$ alkyl$)_2$. Typically a substituted alkyl group has from 1 to 6 substituents. Preferred substituted alkyl groups include trifluoromethyl, $N(C_1\text{-}C_6$ alkyl)H such as $N(CH_3)H$ and $N(C_1\text{-}C_6$ alkyl$)_2$ such as $N(C_2H_5)_2$. Halogen is typically F, Cl, Br or I, preferably F.

An amino group is a $-NH_2$ group and a substituted amino group is typically a -NHR. Typically R is a substituted or unsubstituted alkyl group and preferably contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH and/or halogen. Typically a substituted alkyl group has from 1 to 6 substituents.

In a second aspect the present invention provides compounds of the formula II and pharmaceutically acceptable acid addition salts or quaternary ammonium salts thereof:



in which:

each of Q_2, Q_3, Q_6 and Q_7, which are the same or different, is H, $HNCO(CH_2)_nNR^3R^4$, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of Q_2, Q_3, Q_6 and Q_7 is $HNCO(CH_2)_nNR^3R^4$, and at most three of Q_2, Q_3, Q_6 and Q_7 are $HNCO(CH_2)_nNR^3R^4$ and provided that when Q_2 and Q_6 are both $HNCO(CH_2)_nNR^3R^4$ Q_3 or Q_7 is $HNCO(CH_2)_nR^3R^4$;

each of R^3 and R^4, which are the same or different, is an unsubstituted or substituted alkyl group or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of Q_1, Q_4, Q_5 and Q_8, which are the same or different is H, OH, an amino or substituted amino group, an unsubstituted or substituted alkyl group or halogen.

Preferably, when more than one of Q_2, Q_3, Q_6 and Q_7 is $HNCO(CH_2)_nNR^3R^4$ each group R^3 is the same and each group R^4 is the same.

Preferably, the anthraquinones of formula II contain two $HNCO(CH_2)_nNR^3R^4$ groups. Preferably R^3 and R^4 are methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl or hydroxyethyl. More preferably R^3 and R^4 are the same or R^3 and R^4 together with the nitrogen atom to which they are attached form a heterocyclic group. Preferably the heterocyclic group is a 4 to 8 membered ring, for example a hexamethyleneimino, heptamethyleneimino, azetidino,

pyrrolidino, morpholino or piperidino group which is unsubstituted or substituted with at least one C_1-C_6 alkyl group and/or at least one hydroxy group. More preferably, the heterocyclic group is an unsubstituted hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group or a hydroxymethyl-piperidino group. The heterocyclic group may be a bicyclic ring such as an azabicyclo octano ring, for example 1,3,3-trimethyl-6-azabicyclo[3.2.1]octano. Preferably n is an integer of from 1 to 4, for example 1, 2 or 3, most preferably 2.

If R^3 and R^4 are not the same, preferably at least one of R^3 and R^4 is hydrogen or C_1 to C_6 alkyl. Most preferably at least one of R^3 and R^4 is hydrogen, methyl or ethyl. For example, R^3 is 2-hydroxyethyl and R^4 is ethyl, R^3 is methyl and R^4 is hydrogen, R^3 is $CH_2CH_2N(C_2H_5)_2$ and R^4 is methyl or R^3 is $CH_2CH_2NHCH_3$ and R^4 is methyl.

A substituted or unsubstituted alkyl group typically contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH, halogen, NH_2, $N(C_1$-C_6 alkyl)H and $N(C_1$-C_6 alkyl$)_2$. Typically a substituted alkyl group has from 1 to 6 substitutents. Preferred substituted alkyl groups include trifluoromethyl, $N(C_1$-C_6 alkyl)H such as $N(CH_3)H$ and $N(C_1$-C_6 alkyl$)_2$ such as $N(C_2H_5)_2$. Halogen is typically F, Cl, Br or I, preferably F.

An amino group is a $-NH_2$ group and a substituted amino group is typically a -NHR or $-NR_2$ in which the two groups R may be same or different. Typically R is a substituted or unsubstituted alkyl group and preferably contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH and/or halogen. Typically a substituted alkyl group has from 1 to 6 substituents.

The skilled person will appreciate that the

anthraquinones of the invention are symmetrical and that, for example an anthraquinone of formula (I) in which X_4 and X_8 are both $HNCO(CH_2)_nNR^1R^2$ corresponds to an anthraquinone of formula (I) in which X_1 and X_5 are both $HNCO(CH_2)_nNR^1R^2$, an anthraquinone of formula (I) in which X_4 and X_5 are both $HNCO(CH_2)_nNR^1R^2$ corresponds to an anthraquinone of formula (I) in which X_1 and X_8 are both $HNCO(CH_2)_nNR^1R^2$ and that an anthraquinone of formula (II) in which Q_3 and Q_6 are both $HNCO(CH_2)_nNR^3R^4$ corresponds to an anthraquinone of formula (II) in which Q_2 and Q_7 are both $HNCO(CH_2)_nNR^3R^4$.

Preferably, the anthraquinones of formulae I and II are symmetrical. For example, in anthraquinones of formula I the groups X_1 and X_5, X_2 and X_6, X_3 and X_7 and X_4 and X_8 are the same or the groups X_1 and X_8, X_2 and X_7, X_3 and X_6 and X_4 and X_5 are the same and in anthraquinones of formula II the groups Q_1 and Q_5, Q_2 and Q_6, Q_3 and Q_7 and Q_4 and Q_8 are the same or the groups Q_1 and Q_8, Q_2 and Q_7, Q_3 and Q_6 and Q_4 and Q_5 are the same.

The invention also provides a method for inhibiting the activity of telomerase in a cell in which telomerase is active which comprises adding to the cell or its environment an effective amount of an anthraquinone of formula I or II or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof.

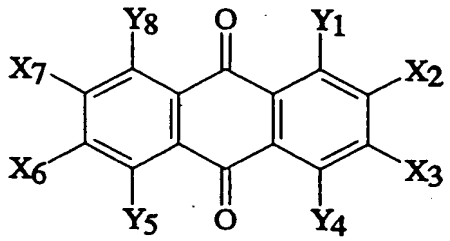
The invention also provides anthraquinones of the formula I or II, a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof or pharmaceutical compositions thereof for use in the treatment of the human or animal body, particularly for the treatment of cancers.

The invention further provides the use of anthraquinones of formula I or II or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof for the manufacture of a medicament for inhibiting the activity of telomerase and/or for treating cancer.

The invention further provides a process for the production of an anthraquinone of formula I or II as defined above which comprises aminolysis of a mono- or bis-(ω-haloalkylcarboxamido)-substituted anthraquinone or, alternatively, acylation of a mono- or diaminoanthraquinone with a ω-aminoalkylalkanoic acid or a derived acylating derivative.

Thus, the present invention provides a process for the production of an anthraquinone of formula I or II, which process comprises:

i) reacting an intermediate of formula B:



(B)

in which:

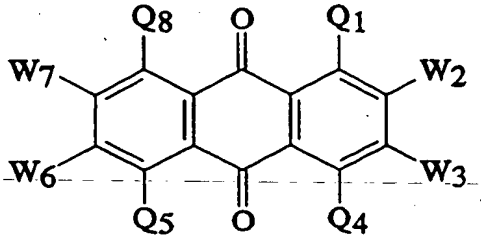
each of Y_1, Y_4, Y_5 and Y_8, which are the same or different, is H, $HNCO(CH_2)_nZ$, OH, an unsubstituted or substituted alkyl group or halogen, provided that at least one of Y_1, Y_4, Y_5 and Y_8 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_6 and X_7 are as defined above for the anthraquinones of formula I;

with the compound of formula (C):

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined above for the anthraquinones of formula I; or

ii) reacting a intermediate of formula (A):



(A)

in which:

each of W_2, W_3, W_5 and W_7, which are the same or different, is H, $HNCO(CH_2)_nZ$, an unsubstituted or substituted alkyl group or halogen, provided that at least one of W_2, W_3, W_6 and W_7 is $HNCO(CH_2)_nZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_4, Q_5 and Q_8 are as defined above for the anthraquinones of formula II;

with a compound of formula (D):

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 are as defined above for the anthraquinone of formula II.

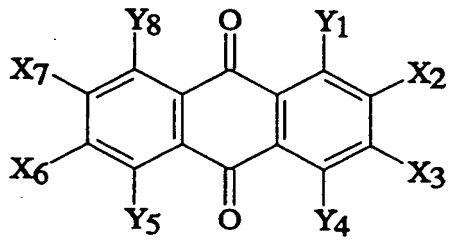
Suitable leaving groups, Z, include halogen, for example F, Cl, Br, I and sulfonate esters of formula $-OSO_2R$ where R is C_{1-6} alkyl, aralkyl or aryl, or other functionalities which can be replaced by aminolysis. Chlorine is a particularly preferred leaving group.

The intermediate of formula (B) can be obtained using the method described in Collier and Neidle, J. Med. Chem., 31: 847-857 (1988). The intermediate of formula (A) can be obtained using the method described in Agbandje *et al.*, J. Med. Chem. 35: 1418-1429 (1992).Further suitable intermediates can be readily obtained using established synthetic procedures for ring-substituted anthraquinones, as described in Bayer, Methoden der Organischen Chemie

7/3c, Verlag, page 111 (1974), and in Zagotto *et al.*, Bioorg. Med. Chem. Lett. 2: 659 (1992). Other anthraquinone derivatives for use as starting materials are available from published synthetic methods, or by ready adaption thereof, or from commercial sources.

The present invention also provides a process for producing anthraquinones of formula (I) in which at least two of X_1, X_4, X_5 and X_8 are $HNCO(CH_2)_nR^1R^2$ and in which at least two of the groups R^1 are not the same and/or at least two of the groups R^2 are not the same, which process comprises:

(i) reacting an intermediate of formula (B'):



(B')

in which:

each of Y_1, Y_4, Y_5 and Y_8, which are the same or different is, H, $HNCO(CH_2)_nZ$, OH, an unsubstituted or substituted alkyl group, an amino or substituted amino group, halogen or NO_2, provided that at least one of Y_1, Y_4, Y_5 and Y_8 is $HNCO(CH_2)_nZ$ and at least one of Y_1, Y_4, Y_5 and Y_8 is NO_2, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_6 and X_7 are as defined above; with a compound of formula (C):

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined above to convert the or each group $HNCO(CH_2)_nZ$ to a group X_1, X_4, X_5 or X_8 which is $HNCO(CH_2)_nNR^1R^2$ as defined in claim 1;

(ii) converting the or each group NO_2 group to an NH_2 group;

(iii) reacting the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, to convert the or each NH_2 group into $HNCO(CH_2)_nZ$;

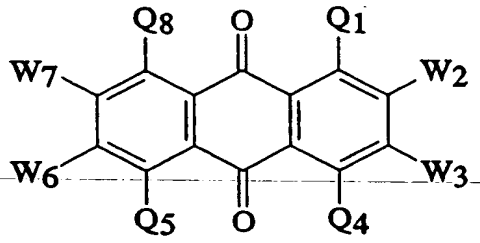
(iv) reacting the product of step (iii) with a compound of formula (C'):

$$R^{1'}R^{2'}NH \qquad (C')$$

wherein $R^{1'}$ and $R^{2'}$ have the same definition as R^1 and R^2 defined above, with the proviso that the compound of formula (C') is not identical to the compound of formula (C) used in step (i), to give a compound of formula (I).

The present invention also provides a process for producing anthraquinones of formula (II) in which at least two of Q_2, Q_3, Q_6 and Q_7 are $HNCO(CH_2)_nR^3R^4$ and in which at least two of the groups R^3 are not the same and/or at least two of the groups R^4 are not the same, which process comprises:

(i) reacting an intermediate of formula (A'):



(A')

in which:

each of W_2, W_3, W_6 and W_7, which are the same or different is, H, $HNCO(CH_2)_nZ$, an unsubstituted or substituted alkyl group, an amino or substituted amino group, halogen or NO_2, provided that at least one of W_2, W_3, W_6 and W_7 is $HNCO(CH_2)_nZ$ and at least one of W_2, W_3, W_6 and W_7 is NO_2, wherein Z is a leaving group and n is an integer of

from 1 to 6, and Q_1, Q_4, Q_5 and Q_8 are as defined above; with a compound of formula (D):

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 are as defined above, to convert the or each group $HNCO(CH_2)_nZ$ to a group Q_2, Q_3, Q_6 or Q_7 which is $HNCO(CH_2)_nNR^3R^4$ as defined above;

(ii) converting the or each group NO_2 group to an NH_2 group;

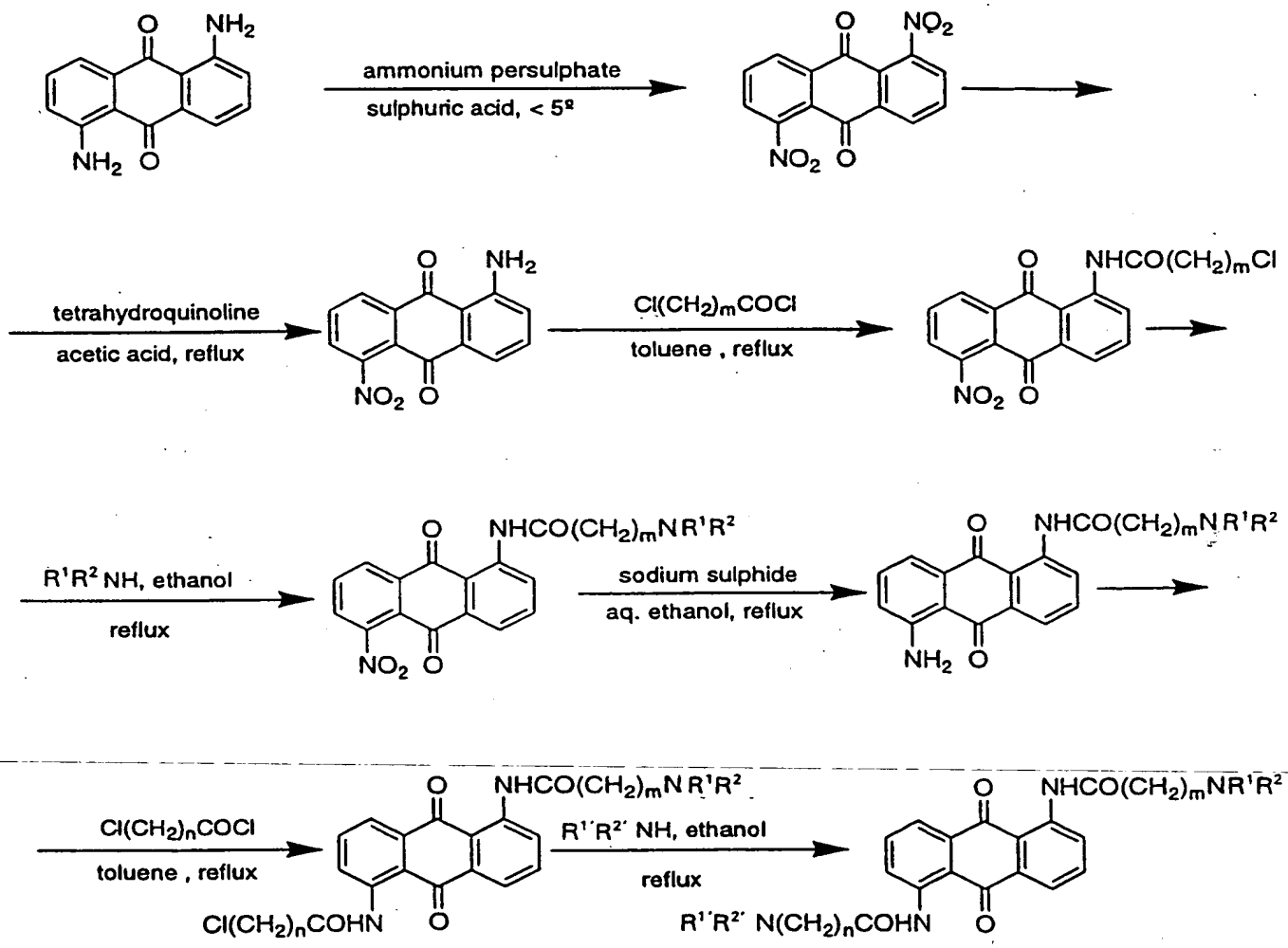
(iii) reacting the product of step (ii) with $Z(CH_2)_n$ COZ wherein Z is a leaving group and n is an integer of from 1 to 6, to convert the or each NH_2 group into $HNCO(CH_2)_nZ$;

(iv) reacting the product of step (iii) with a compound of formula (D'):

$$R^{3'}R^{4'}NH \qquad (D')$$

wherein $R^{3'}$ and $R^{4'}$ have the same definition as R^3 and R^4 defined above, with the proviso that the compound of formula (D') is not identical to the compound of formula (D) used in step (i), to give a compound of formula (I).

Anthraquinones of formula (I) in which two groups R^1 are not the same and/or two groups R^2 are not the same may be produced in accordance with the following reaction scheme.


ammonium persulphate
sulphuric acid, < 5º
tetrahydroquinoline
acetic acid, reflux
Cl(CH2)mCOCl
toluene , reflux
R1R2 NH, ethanol
reflux
sodium sulphide
aq. ethanol, reflux
Cl(CH2)nCOCl
toluene , reflux
R1'R2' NH, ethanol
reflux

wherein the definition of $R^{1'}$ and $R^{2'}$ is the same as that for R^1 and R^2 above (with the proviso that $R^{1'}$ is not the same as R^1 and/or $R^{2'}$ is not the same as R^2).

The skilled person will appreciate that other anthraquinones of formula (I) in which two groups R^1 are not the same and/or two groups R^2 are not the same may be made by analogous reaction schemes.

The skilled person will also appreciate that anthraquinones of formula (II) in which two groups R^3 are not the same and/or two groups R^4 are not the same may be made in an analogous manner.

The invention provides a process for the production of a salt of an anthraquinone of formula I or II as defined above by subsequent alkylation treatment of a precursor anthraquinone of formula I or II, preferably with an alkyl halide or aralkyl halide, to form the corresponding quaternary ammonium halide salt.

Physiologically acceptable salts according to the invention which may be conveniently used include physiologically acceptable acid addition salts, including the hydrochloride, acetate, maleate and, in particular, quaternary (eg methyl or ethyl iodide) salts. Preferred quaternary salts of compounds of formula I or II include those in which $-N^+R^1R^2R^9X^-$ or $-N^+R^3R^4R^9X^-$ have the same NR^1R^2 or NR^3R^4 substituent groups and R^9 is $-CH_3$ or $-CH_2CH_3$ and X^- is a iodide or physiologically acceptable anion.

Acid addition salts according to the invention include mono- and di-carboxylic acids in which the non-carbonyl moiety of the carboxylate grouping is selected from straight or branched chain alkyl (e.g. methyl, n-propyl, n-butyl or t-butyl); cyclic alkyl (e.g. cyclohexyl); alkoxyalkyl (e.g. methoxymethyl), carboxyalkyl (e.g. carboxyethyl), aralkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy

or amino); sulfonic acids such as alkyl- or aralkyl-sulfonate (e.g. methanesulfonate); mono- or di-phosphoric acids which may or may not be blocked, amino acids (e.g. L-valine or L-isoleucine) and nitrates. With regard to these acid components, unless otherwise specified, any alkyl moieties present in such acids preferably contain 1 to 18 carbon atoms, particularly 1 to 4 carbon atoms, in the case of straight chain alkyl groups, or 3 to 7 carbon atoms in the case of branched or cyclic alkyl groups. Any aryl moiety present in such acids advantageously comprises a phenyl group.

Any reference herein to any of the above compounds of the invention also includes a reference to a physiologically acceptable salt thereof.

Particularly preferred compounds of the invention include 1,5-substituted compounds, that is compounds of formula I wherein X_1 and X_5 are both $HNCO(CH_2)_nNR^1R^2$, 1,8-substituted compounds, that is compounds of formula I wherein X_1 and X_8 are both $HNCO(CH_2)_nR^1R^2$, and 2,7-substituted compounds, that is compounds of formula II wherein Q_2 and Q_7 are both $HNCO(CH_2)_nNR^3R^4$ and pharmaceutically acceptable acid addition salts or quaternary ammonium salts thereof. Preferred anthraquinones of formula I include:

1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
1,5-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
1,5-Bis(3-morpholinopropionamido)anthracene-9,10-dione;
1,5-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
1,5-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
1,8-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione;

1,8-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
1,8-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione;
Preferred salts of anthraquinones of formula I include:
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt;
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide;
1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione maleate salt.

Preferred anthraquinones of formula II include:
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
2,7-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
2,7-Bis(3-morpholinopropionamido)anthracene-9,10-dione;
2,7-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
2,7-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione.

Preferred salts of anthraquinones of formula II include:
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione maleate salt;
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide.

The anthraquinones of formula I or II may be used *in vitro* or *in vivo* as telomerase inhibitors. For *in vitro* use, the compounds will be useful in the development and standardization of assays for telomerase and inhibitors thereof and in gene probe-based applications, or biological/molecular biological applications, for example

microscopy. For example, in a preferred assay format described herein, telomerase is obtained from a partial purification of a mammalian cell extract. In order to standardize the activity of the assay or results for telomerase inhibitors using the assay, compounds of the invention may be used, e.g. those compounds which have already been used in previous assays of the same format using different cell extracts.

For *in vivo* use the assays will be used in methods of treatment of uncontrolled cell proliferation, particularly cancers. Such cancers include leukaemias, small cell and non-small cell lung cancer, ovarian, breast, gastric, liver, cervical, colorectal, bladder, renal, stomach, brain, prostate, testicular, head and neck, skin and thyroid cancers, melanomas, non-Hodgkin's lymphoma, leukaemias, sarcomas and neuro-blastoma.

Because the inhibition of telomerase activity in a cell will not necessarily lead to cell death immediately the anthraquinones of formula I or II may be relatively slow acting. In view of this these compounds may be used as a single agent or in combination with other anti-cancer compounds, particularly cytotoxic compounds such as doxorubicin, cisplatin, or other anti-cancer treatments such as radiation, ADEPT (antibody-directed enzyme prodrug therapy), VDEPT (vector-directed enzyme prodrug therapy), and GDEPT (gene-directed enzyme prodrug therapy).

For example, a patient may first be treated with another anti-cancer compound or treatment which will destroy a substantial portion of the cancer. Alternatively, a patient may be treated simultaneously with another anti-cancer compound or treatment which will destroy a substantial portion of the cancer. In order to treat or control the regrowth of any residual primary tumour cells which may be resistant to the main therapy, anthraquinones of formula I or II may be administered to

the patient over prolonged periods of time.

Such chronic administration may also be appropriate to prevent or treat secondary tumours in the event that metastatic spread of the primary tumour occurs. Anthraquinones of formula I or II may also be used in conjunction with other compounds designed to prevent or treat metastases, particularly matrix metalloproteinase inhibitors (MMIs).

Combined therapy with second compounds such as MMIs will be particularly advantageous since the second compound(s) can target a separate locus within the tumour cell, for example in the case of MMIs the enzymes responsible for invasion of the tumour cells. In this manner the tumour cells may be prevented from spreading for sufficient time such to inhibit telomerase activity for long enough to allow the cells to differentiate and/or senesce.

The anthraquinones of formula I or II may be administered to mammals including humans by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient.

For each of the above-indicated utilities and indications the amount required of the individual active ingredients will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, for each of these utilities and indications, a suitable, effective dose will be in the range 0.01 to 50 mg per kilogram body weight of recipient per day,

preferably in the range 0.01 to 20 mg per kilogram body weight per day and most preferably in the range 0.01 to 10 mg per kilogram body weight per day (unless otherwise indicated all weights of active ingredient are calculated as the parent compound; for salts thereof the figures would be increased proportionately.)

The desired dose may if desired be presented as two, three, four or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 0.1 to 3000 mg, preferably 0.1 to 650 mg of active ingredient per unit dosage form.

Doses of compounds of the invention may be administered at sub-daily, or daily intervals, or less frequently, for example on alternate days, weekly or fortnightly. In general the doses will be the same as the above daily dose although higher doses, particularly when formulated to be released over a prolonged period of time, may be used.

While it is possible for the compounds to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be

prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent.

A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with one or more additives. Examples of suitable additives include binders such as povidone; gelatin, lubricants, inert diluents and disintegrants as for tablets.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain

the active compound 1) in an optionally buffered, aqueous solution or 2) dissolved in an adhesive or 3) dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Where anthraquinones of the formula I or II are used in conjunction with second anti-cancer compounds, the active ingredient(s) and pharmacologically active agents may be administered together or separately and, when administered separately this may occur simultaneously or sequentially in any order. The amounts of the active ingredient(s) and pharmacologically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

The anthraquinones of formula I or II may be produced by various methods known in the art of organic chemistry in general. Starting materials are either known and readily available from commercial sources or may themselves be produced by known and conventional techniques.

The following examples illustrate the invention. For

the purposes of clarity, the examples are presented in two sections; section A illustrates the synthesis of anthraquinones of formula I or II and salts thereof, and section B illustrates the biological assays of compounds of the invention.

Section A - Preparative Methods

Preparative method for anthraquinone free bases of formula I and salts thereof:

Example 1

1,5-Bis(3-chloropropionamido)anthracene-9,10-dione BSU-9007

To a stirred suspension of 1,5-diaminoanthraquinone (3.0 g, 12.6 mmol) and pyridine (0.5 ml) in toluene (500 ml) at 70 °C was added dropwise 3-chloropropanoyl chloride (5.0 ml, 57 mmol) in toluene (50 ml). The mixture was stirred at 70 °C for 6 hours and cooled to room temperature. The mixture was filtered, washed with DCM (50 ml) and the combined filtrate evaporated to yield the crude product as a brown solid. Recrystallisation from DMF-EtOH (4:1 v/v) afforded chloroamide **BSU-9007** (3.0 g, 57%) as yellow/brown crystals; mp 280-281 °C; NMR δ($CDCl_3$) 3.04 (4H, t, J = 6.4, $COCH_2$), 3.95 (4H, t, J = 6.4, CH_2Cl), 7.82 (2H, t, J = 8.1, H-3,7), 8.08 (2H, dd, J = 8.1, 1.0, H-2,6), 9.16 (2H, dd, J = 8.1, 1.0, H-4,8), 12.40 (2H, s, NH); MS (rel intensity) m/z 421 (100), 419 (74), 418 (20), 411 (25), 403 (23), 383 (52), 357 (26), 344 (25), 293 (26); Calcd ($[M+1]^+$) 419.0565. Found 419.0575; Anal. Calcd ($C_{20}H_{16}N_2O_4Cl_2$): C, 57.30; H, 3.85; N, 6.68; Cl, 16.91. Found C, 57.53; H, 4.09; N, 6.77; Cl, 16.86.

Example 2

1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione BSU-9009.

General aminolysis procedure

To a stirred refluxing suspension of 1,5-bis(3-chloropropionamido)anthracene-9,10-dione **BSU-9007** (1.00 g, 2.4 mmol) and NaI (0.3 g) in EtOH (40 ml) was added dropwise piperidine (3.0 ml, 30 mmol) in EtOH (10 ml). The mixture was stirred at reflux for 3 hours, cooled to 0 °C,

filtered and washed with ether (50 ml). The crude solid was dissolved in hot chloroform (150 ml) and treated with decolourising charcoal, filtered and the filtrate evaporated to yield an orange solid. Recrystallisation from DMF-EtOH (9:1 v/v) afforded amide **BSU-9009** (1.1 g, 89%) as orange needles; mp 214-215 °C; NMR $\delta(CDCl_3)$ 1.47 (4H, m, $(CH_2CH_2)_2CH_2$), 1.63 (8H, m, $N(CH_2CH_2)_2$), 2.52 (8H, t, J = 5.0, $N(CH_2CH_2)_2$), 2.72 (4H, m, $COCH_2CH_2$), 2.86 (4H, m, $COCH_2$), 7.76 (2H, t, J = 8.0, H-3,7), 8.03 (2H, dd, J = 8.0, 1.0, H-2,6), 9.10 (2H, dd, J = 8.0, 1.0, H-4,8), 12.31 (2H, s, NH); MS (rel intensity) m/z 517 (100), 516 (7), 307 (43), 289 (40), 246 (100), 207 (22); Calcd ($[M+1]^+$) 517.2815. Found 517.2825; Anal. Calcd ($C_{30}H_{36}N_4O_4$): C, 69.74; H, 7.02; N, 10.84. Found C, 69.50; H, 7.04; N, 10.77.

Example 3

1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt BSU-9010.

General Procedure

A solution of amino amide **BSU-9009** (0.56 g, 1 mmol) in glacial acetic acid (6 ml) was heated at 50-60 °C for 30 min, treated with decolourising charcoal and filtered. The filtrate was triturated with dry ether, filtered and the precipitate repeatedly washed with dry ether and dried *in vacuo* at 25 °C to give diacetate **BSU-9010** (0.61 g, 96%) as an orange solid. mp 215 °C.

Example 4

1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide BSU-9011.

General Procedure

A mixture of amino amide **BSU-9009** (0.56 g, 1 mmol) and iodomethane (3.3 ml, 50 mmol) in acetone (20 ml) was stirred at room temperature for 24 h. The resulting

mixture was filtered, washed with dry ether and dried *in vacuo* at 25 °C to give dimethiodide **BSU-9011** (0.78 g, 97.5%) as an orange solid. mp 230 °C dec. Anal. Calcd ($C_{32}H_{42}N_4O_4I_2.H_2O$): C, 46.96; H, 5.42; N, 6.84; I, 31.01. Found C, 47.17; H, 5.14; N, 6.80; I, 31.04.

Example 5

1,5-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione BSU-9012.

Chloroamide **BSU-9007** was treated with pyrrolidine according to the general aminolysis procedure to give amide **BSU-9012** (1.4 g, 80%) as yellow/orange needles; mp 194-195 °C; NMR $\delta(CDCl_3)$ 1.85 (8H, m, $N(CH_2CH_2)_2$), 2.66 (8H, m, $N(CH_2CH_2)_2$), 2.76 (4H, t, J = 7.6, $COCH_2CH_2$), 2.96 (4H, t, J = 7.6, $COCH_2$), 7.77 (2H, t, J = 8.0, H-3,7), 8.04 (2H, d, J = 8.0, H-2,6), 9.13 (2H, d, J = 8.0, H-4,8), 12.39 (2H, s, NH); MS (rel intensity) m/z 489 (100), 488 (39); Calcd ($[M+1]^+$) 489.2502. Found 489.2512; Anal. Calcd ($C_{28}H_{32}N_4O_4$): C, 68.83; H, 6.6; N, 11.47. Found C, 68.70; H, 6.61; N, 11.49. Diacetate salt (**BSU-9013**), mp 135-136 °C; Dimethiodide, (**BSU-9014**), mp 238 °C dec. Anal. Calcd ($C_{30}H_{38}N_4O_4I_2.H_2O$): C, 45.58; H, 5.1; N, 7.09; I, 32.11. Found C, 45.79; H, 5.01; N, 7.04; I, 32.13.

Example 6

1,5-Bis(3-morpholinopropionamido)anthracene-9,10-dione BSU-9015.

Chloroamide **BSU-9007** was treated with morpholine according to the general aminolysis procedure to give amide **BSU-9015** (1.6 g, 85%) as yellow needles; mp 268 °C; NMR $\delta(CDCl_3)$ 2.59 (8H, m, $N(CH_2CH_2)_2O$), 2.72 (4H, t, J = 6.0 $COCH_2CH_2$), 2.89 (4H, t, J = 6.0 $COCH_2$), 3.76 (8H, t, J = 4.6, $N(CH_2CH_2)_2O$), 7.79 (2H, t, J = 8.0, H-3,7), 8.04 (2H, d, J = 8.0, H-2,6), 9.11 (2H, d, J = 8.0, H-4,8), 12.37 (2H, s, NH); MS (rel intensity) m/z 521 (100), 520 (30);

Calcd ([M+1]$^+$) 521.2400. Found 521.2410; Anal. Calcd ($C_{28}H_{32}N_4O_6$): C, 64.6; H, 6.2; N, 10.76. Found C, 64.4; H, 6.14; N, 10.65. Diacetate salt (**BSU-9016**), mp 266 °C; Dimethiodide, (**BSU-9017**), mp 245 °C dec. Anal. Calcd ($C_{30}H_{38}N_4O_6I_2$): C, 44.79; H, 4.76; N, 6.96; I, 31.55. Found C, 45.15; H, 4.73; N, 6.91; I, 34.14.

Example 7

1,5-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione BSU-9018.

Chloroamide **BSU-9007** was treated with dimethylamine (10 ml of a 5.6M solution in EtOH) according to the general aminolysis procedure to give amide **BSU-9018** (1.30 g, 83%) as yellow needles; mp 176-177 °C; NMR δ($CDCl_3$) 2.36 (12H, s, CH_3), 2.69 (4H, m, $COCH_2CH_2$), 2.84 (4H, m, $COCH_2$), 7.77 (2H, t, J = 8.0, H-3,7), 8.04 (2H, d, J = 8.0, H-2,6), 9.14 (2H, d, J = 8.0, H-4,8), 12.39 (2H, s, NH); MS (rel intensity) *m/z* 437 (100), 436 (27), 307 (30), 289 (17); Calcd ([M+1]$^+$) 437.2189. Found 437.2179; Anal. Calcd ($C_{24}H_{28}N_4O_4$): C 66.04; H 6.47; N 12.84. Found C 66.02; H 6.43; N 12.76. Diacetate salt (**BSU-9019**), mp 142-143 °C; Dimethiodide, (**BSU-9020**), mp 250 °C dec. Anal. Calcd ($C_{26}H_{34}N_4O_4I_2.0.5H_2O$): C 42.81; H 4.84; N 7.68; I 34.8. Found C 42.87; H 4.94; N 7.49; I 35.68.

Example 8

1,5-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione BSU-9021.

Chloroamide **BSU-9007** was treated with diethylamine according to the general aminolysis procedure to give amide **BSU-9021** (1.28 g, 72%) as orange crystals; mp 174-175 °C; NMR δ($CDCl_3$) 1.08 (12H, t, J = 7.0, CH_3), 2.65 (12H, m, J = 7.0, NCH_2), 2.97 (4H, t, J = 7.0, $COCH_2$), 7.76 (2H, t, J = 8.0, H-3,7), 8.04 (2H, d, J = 8.0, H-2,6), 9.13 (2H, d, J = 8.0, H-4,8), 12.33 (2H, s, NH); MS (rel

intensity) *m/z* 493 (100), 492 (36); Calcd ([M+1]$^+$) 493.2815. Found 493.2825; Anal. Calcd ($C_{28}H_{36}N_4O_4.0.5H_2O$): C 67.04; H 7.43; N 11.17. Found C 67.01; H 7.22; N 11.10. Diacetate salt (**BSU-9022**), mp 91 °C; Dimethiodide, (**BSU-9023**), mp 235 °C dec. Anal. Calcd ($C_{30}H_{42}N_4O_4I_2.0.5H_2O$): C 45.87; H 5.52; N 7.13; I 32.31. Found C 45.84; H 5.49; N 6.99; I 33.01.

Example 9

1,8-Diaminoanthracene-9,10-dione BSU-3300

A stirred mixture of 1,8-dichloroanthracene-9,10-dione (41.6 g, 0.15 mol), phthalimide (52.7 g, 0.385 mol), anhydrous sodium acetate (29.6 g, 0.361 mol) and nitrobenzene (77 ml) was heated to 180 °C. Quinoline (25 ml) and copper powder (300 mesh, 0.72 g) were added and the mixture heated at 200 °C for 1 hour. The reaction mixture was allowed to cool and left to stand overnight. The mixture was filtered and washed with nitrobenzene (3 x 100 ml), ethanol (3 x 100 ml), hot water (3 x 200 ml), ethanol (2 x 100 ml), ether (2 x 100 ml) and dried to give the intermediate diphthalimide as a pale yellow/orange solid; mp > 360 °C (56.66 g, 76%). The crude solid (56.0 g) was added to conc. H_2SO_4 (400 ml) and the mixture heated to 95 °C with stirring for 45 mins. The reaction mixture was cooled to 5 °C and crushed ice (150 g) was slowly added. The reaction mixture was poured onto ice/water (1.5 L) with stirring. The resulting precipitate was collected by filtration and washed with water until neutral and dried *in vacuo*. Recrystallisation from ethanol afforded the product as red/purple needles (27.0 g, 98%); mp 270-271 °C; NMR δ(DMSO) 7.15 (2H, dd, *J* = 8.5, 1.4, H-2,7), 7.34 (2H, dd, *J* = 7.4, 1.4, H-4,5), 7.45 (2H, dd, *J* = 8.5, 7.4, H-3,6), 7.86 (4H, br s, NH_2); MS (rel intensity) *m/z* 238 (100), 210 (12), 181 (7), 154 (8), 119 (9), 91 (7), 77 (8); Anal. Calcd ($C_{14}H_{10}N_2O_2$): C, 70.58; H,

4.23; N, 11.75. Found C, 70.40; H, 4.22; N, 11.70.

Example 10

1,8-Bis(3-chloropropionamido)anthracene-9,10-dione BSU-9040

To a stirred suspension of 1,8-diaminoanthraquinone **BSU-3300** (3.0 g, 12.6 mmol) and pyridine (0.5 ml) in toluene (500 ml) at 70 °C was added dropwise 3-chloropropanoyl chloride (5.0 ml, 57 mmol) in toluene (50 ml). The mixture was stirred at 70 °C for 6 hours and cooled to room temperature. The mixture was filtered, washed with DCM (50 ml) and the combined filtrate evaporated to yield the crude product as a red solid. Recrystallisation from DMF-EtOH (2:1 v/v) afforded chloroamide **BSU-9040** (3.7 g, 70%) as orange crystals; mp 249-250 °C; NMR δ($CDCl_3$) 3.06 (4H, t, J = 6.5, $COCH_2$), 3.97 (4H, t, J = 6.5, CH_2Cl), 7.81 (2H, t, J = 8.5, H-3,6), 8.08 (2H, dd, J = 8.5, 1.0, H-2,7), 9.18 (2H, dd, J = 8.5, 1.0, H-4,5), 12.18 (2H, s, NH); MS (rel intensity) *m/z* 418 (21), 382 (21), 347 (13), 328 (34), 292 (25), 265 (55), 238 (90), 91 (18), 63 (46), 55 (100); Anal. Calcd ($C_{20}H_{16}N_2O_4Cl_2$): C, 57.30; H, 3.85; N, 6.68; Cl, 16.91. Found C, 57.55; H, 3.84; N, 6.74; Cl, 16.98.

Example 11

1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione BSU-9041.

General aminolysis procedure

To a stirred refluxing suspension of 1,8-bis(3-chloropropionamido)anthracene-9,10-dione **BSU-9040** (1.50 g, 3.6 mmol) and NaI (0.3 g) in EtOH (80 ml) was added dropwise piperidine (4.5 ml, 30 mmol) in EtOH (10 ml). The mixture was stirred at reflux for 4 hours, cooled to 0 °C, filtered and washed with ether (50 ml). The crude solid was dissolved in hot chloroform (150 ml) and treated with

decolourising charcoal, filtered and the filtrate evaporated to yield a yellow solid. Recrystallisation from DMF-EtOH (9:1 v/v) afforded amide **BSU-9041** (1.64 g, 89%) as yellow needles; mp 183-184 °C; NMR $\delta(CDCl_3)$ 1.50 (4H, m, $(CH_2CH_2)_2CH_2$), 1.65 (8H, m, $N(CH_2CH_2)_2$), 2.57 (8H, m, $N(CH_2CH_2)_2$), 2.83 (4H, t, J = 5.6 $COCH_2CH_2$), 2.88 (4H, t, J = 5.6 $COCH_2$), 7.77 (2H, t, J = 8.0, H-3,6), 8.05 (2H, dd, J = 8.0, 1.0, H-2,7), 9.12 (2H, dd, J = 8.0, 1.0, H-4,5), 12.11 (2H, s, NH); MS (rel intensity) *m/z* 517 (31), 431 (14), 405 (9), 376 (32), 347 (14), 292 (10), 265 (8), 238 (17), 138 (100), 112 (32); Anal. Calcd ($C_{30}H_{36}N_4O_4.1.2H_2O$): C, 66.94; H, 7.19; N, 10.41. Found C, 66.90; H, 6.81; N, 10.44.

Example 12

1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt BSU-9042.

General Procedure

A solution of amino amide **BSU-9041** (0.516 g, 1 mmol) in glacial acetic acid (6 ml) was heated at 50-60 °C for 45 min, treated with decolourising charcoal and filtered. The filtrate was triturated with dry ether, filtered and the precipitate repeatedly washed with dry ether and dried *in vacuo* at 25 °C to give diacetate **BSU-9042** (0.47 g, 74%) as an orange solid. mp 174-176 °C.

Example 13

1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide BSU-9043.

General Procedure

A mixture of amino amide **BSU-9041** (0.516 g, 1 mmol) and iodomethane (3.3 ml, 50 mmol) in dichloromethane (25 ml) was stirred at room temperature for 24 h. The resulting mixture was filtered, washed with dry ether and dried *in vacuo* at 25 °C to give dimethiodide **BSU-9043**

(0.74 g, 92.5%) as an orange solid, mp 244 °C dec. Anal. Calcd ($C_{32}H_{42}N_4O_4I_2.2.5H_2O$): C, 45.46; H, 5.60; N, 6.63; I, 30.02. Found C, 45.21; H, 5.03; N, 6.54; I, 30.33.

Example 14

1,8-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione BSU-9044.

Chloroamide **BSU-9040** was treated with pyrrolidine according to the general aminolysis procedure to give amide **BSU-9044** (1.07 g, 61%) as yellow needles; mp 184-186 °C; NMR δ($CDCl_3$) 1.84 (8H, m, $N(CH_2CH_2)_2$), 2.67 (8H, m, $N(CH_2CH_2)_2$), 2.80 (4H, m, $COCH_2CH_2$), 2.99 (4H, m, $COCH_2$), 7.77 (2H, t, J = 8.0, H-3,6), 8.05 (2H, d, J = 8.0, H-2,7), 9.14 (2H, d, J = 8.0, H-4,5), 12.17 (2H, s, NH); MS (rel intensity) m/z 489 (9), 417 (10), 391 (8), 362 (19), 347 (18), 292 (13), 238 (19), 155 (17), 124 (100); Anal. Calcd ($C_{28}H_{32}N_4O_4$): C, 68.83; H, 6.6; N, 11.47. Found C, 68.68; H, 6.47; N, 11.34. Diacetate salt (**BSU-9045**), mp 179-180 °C; Dimethiodide, (**BSU-9046**), mp 228-230 °C dec. Anal. Calcd ($C_{30}H_{38}N_4O_4I_2.2H_2O$): C, 44.57; H, 5.24; N, 6.93; I, 31.39. Found C, 44.34; H, 5.18; N, 6.77; I, 31.72.

Example 15

1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione BSU-9047.

Chloroamide **BSU-9040** was treated with morpholine according to the general aminolysis procedure except the mixture was heated at reflux for 24 hours to give amide **BSU-9047** (1.82 g, 97%) as an orange solid; mp 230 °C; NMR δ($CDCl_3$) 2.58 (8H, t, J = 4.4, $N(CH_2CH_2)_2O$), 2.75 (4H, t, J = 6.6, $COCH_2CH_2$), 2.88 (4H, t, J = 6.6, $COCH_2$), 3.74 (8H, t, J = 4.4, $N(CH_2CH_2)_2O$), 7.76 (2H, t, J = 7.8, H-3,6), 8.04 (2H, dd, J = 7.8, 1.0, H-2,7), 9.13 (2H, dd, J = 7.8, 1.0, H-4,5), 12.05 (2H, s, NH); MS (rel intensity) m/z 521 (100), 329 (12), 307 (45), 289 (27); Calcd ($[M+1]^+$)

521.2400. Found 521.2420; Anal. Calcd ($C_{28}H_{32}N_4O_6$): C, 64.6; H, 6.2; N, 10.76. Found C, 64.52; H, 5.99; N, 10.54. Dimethiodide, (**BSU-9049**), mp 232-233 °C dec. Anal. Calcd ($C_{30}H_{38}N_4O_6I_2.2H_2O$): C, 42.87; H, 5.04; N, 6.67; I, 30.20. Found C, 42.89; H, 4.83; N, 6.41; I, 29.01.

Example 16

1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione maleate salt BSU-9048.

General Procedure

A solution of amino amide **BSU-9047** (0.52 g, 1 mmol) in $CHCl_3$ (25 ml) was added a solution of maleic acid (0.116 g, 1 mmol) in MeOH7 (4 ml) and the solution stirred at room temperature for 30 minutes. Ether (25 ml) was added slowly, and the resulting precipitate filtered, washed with dry ether and dried *in vacuo* at 25 °C to give the maleate **BSU-9048** (0.60 g, 94%) as an orange solid. mp 190-192 °C.

Example 17

1,8-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione BSU-9050.

Chloroamide **BSU-9040** was treated with dimethylamine (10 ml of a 5.6M solution in EtOH) according to the general aminolysis procedure to give amide **BSU-9050** (1.20 g, 76%) as orange needles; mp 126 °C; NMR δ($CDCl_3$) 2.36 (12H, s, CH_3), 2.70 (4H, m, $COCH_2CH_2$), 2.80 (4H, m, $COCH_2$), 7.75 (2H, t, J = 8.0, H-3,6), 8.03 (2H, dd, J = 8.0, 1.0, H-2,7), 9.13 (2H, dd, J = 8.0, 1.0, H-4,5), 12.19 (2H, s, NH); MS (rel intensity) m/z 437 (100), 365 (15), 338 (15); Calcd ([M+1]$^+$) 437.2189. Found 437.2170; Anal. Calcd ($C_{24}H_{28}N_4O_4$): C 66.04; H 6.47; N 12.83. Found C 65.92; H 6.34; N 12.80. Maleate salt (**BSU-9051**), mp 188-189 °C; Dimethiodide, (**BSU-9052**), mp 263 °C dec. Anal. Calcd ($C_{26}H_{34}N_4O_4I_2.H_2O$): C, 42.29; H, 4.91; N, 7.59; I, 34.37.

Found C, 42.00; H, 4.62; N, 7.39; I, 32.62.

Example 18

1,8-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione BSU-9053.

Chloroamide **BSU-9040** was treated with diethylamine according to the general aminolysis procedure to give amide **BSU-9053** (1.58 g, 89%) as orange crystals; mp 175-176 °C; NMR $\delta(CDCl_3)$ 1.08 (12H, t, J = 7.0, CH_3), 2.66 (12H, m, J = 7.0, NCH_2), 2.97 (4H, t, J = 7.0, $COCH_2$), 7.75 (2H, t, J = 8.0, H-3,6), 8.04 (2H, dd, J = 8.0, 1.0, H-2,7), 9.13 (2H, dd, J = 8.0, 1.0, H-4,5), 12.11 (2H, s, NH); MS (rel intensity) m/z 493 (100), 307 (28), 289 (18); Calcd ($[M+1]^+$) 493.2815. Found 493.2800; Anal. Calcd ($C_{28}H_{36}N_4O_4.3.75H_2O$): C, 60.04; H, 7.83; N, 10.00. Found C, 60.04; H, 6.40; N, 10.01. Maleate salt (**BSU-9054**), mp 149-150 °C; Dimethiodide, (**BSU-9055**), mp 218-220 °C. Anal. Calcd ($C_{30}H_{42}N_4O_4I_2.6H_2O$): C, 40.73; H 6.15; N 6.33; I 28.69. Found C 41.01; H 4.83; N 6.38; I 28.24.

Preparative method for anthraquinone free bases of formula II and acid addition salts thereof:

Example 19

2,7-Dinitroanthracene-9,10-dione BSU-3301

Anthrone (21.25 g, 0.109 mol) was added with stirring to a cooled solution of fuming nitric acid (142 ml) at such a rate as to maintain a reaction temperature of 5 °C. After completion of the addition (*ca.* 1.5 hours) the reaction mixture was allowed to reach ambient temperature. The reaction mixture was poured into a cooled solution of glacial acetic acid (430 ml), lightly stoppered and allowed to stand at room temperature for 1 week. The resulting precipitate was collected by filtration, washed with glacial acetic acid (3 x 25 ml), hexane (3 x 25 ml)

and dried. The crude solid was suspended in glacial acetic acid (4 L) and heated at reflux until the evolution of nitrous fumes had ceased (*ca.* 2 hours). The mixture was allowed to cool to room temperature and left to stand for 48 hours. The resulting precipitate was collected by filtration, washed with glacial acetic acid (3 x 30 ml), hexane (3 x 30 ml) and dried to give a pale yellow solid (10.34 g, 32%). Recrystallisation from nitrobenzene/glacial acetic acid (1:1) afforded a pure sample of **BSU-3301**, mp 290-291 °C; NMR δ(DMSO) 8.48 (2H, dd, J = 8.4, 1.4, H-4,5), 8.71 (2H, dt, J = 8.4, 1.9, H-3,6), 8.83 (2H, t, J = 1.9, H-1,8); MS (rel intensity) *m/z* 298 (100), 252 (75), 196 (22), 178 (23), 150 (67), 75 (34); Anal. Calcd ($C_{14}H_6N_2O_6$): C, 56.39; H, 2.03; N, 9.39;. Found C, 56.28; H, 2.14; N, 9.09.

Example 20

2,7-Diaminoanthracene-9,10-dione BSU-3303

To a stirred suspension of 2,7-dinitroanthracene-9,10-dione **BSU-3301** (9.4 g, 31.5 mmol) in ethanol (340 ml) was added a solution of sodium sulphide nonahydrate (34.1 g, 142 mmol) and sodium hydroxide (13.5 g, 338 mmol) in water (590 ml). The mixture was heated at reflux for 6 hours and left to stand overnight. The ethanol was removed *in vacuo* and the residue cooled to 0-5 °C. The resulting precipitate was collected by filtration, repeatedly washed with water and dried. Recrystallisation from ethanol/water afforded the product as an orange/red solid (7.35 g, 98%); mp 337-338 °C; NMR δ_(DMSO) 6.42 (4H, br s, NH_2) 6.89 (2H, dd, J = 8.5, 1.5, H-3,6), 7.23 (2H, d, J = 1.5, H-1,8), 7.84 (2H, d, J = 8.5, H-4,5); Anal. Calcd ($C_{14}H_{10}N_2O_2$): C, 70.58; H, 4.23; N, 11.76;. Found C 70.54; H 4.16; N 11.56.

Example 21

2,7-Bis(3-chloropropionamido)anthracene-9,10-dione BSU-

3304

A stirred suspension of 2,7-diaminoanthraquinone **BSU-3303** (3.0 g, 12.6 mmol) and 3-chloropropanoyl chloride (60 ml) was heated at reflux for 4 hours. The mixture was cooled to 0 °C and filtered. The crude solid was washed with dry ether (4 x 25 ml), toluene (25 ml) and again with dry ether (25 ml) to give the product **BSU-3304** (4.33 g, 82%) as a yellow solid; mp 289-290 °C dec; NMR δ(DMSO) 2.92 (4H, t, J = 6.1, $COCH_2$), 3.91 (4H, t, J = 6.1, CH_2Cl), 8.05 (2H, dd, J = 8.5, 2.0, H-3,6), 8.17 (2H, d, J = 8.5, H-4,5), 8.48 (2H, d, J = 2.0, H-1,8), 10.72 (2H, s, NH); Anal. Calcd ($C_{20}H_{16}N_2O_4Cl_2.0.25H_2O$): C, 56.69; H, 3.92; N, 6.74; Cl, 16.73. Found C, 56.46; H, 3.75; N, 6.50; Cl, 16.78.

Example 22

2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione BSU-9056.

General aminolysis procedure

To a stirred refluxing suspension of 2,7-bis(3-chloropropionamido)anthracene-9,10-dione **BSU-3304** (1.50 g, 3.6 mmol) and NaI (0.3 g) in EtOH (70 ml) was added dropwise piperidine (4.5 ml, 30 mmol) in EtOH (15 ml). The mixture was stirred at reflux for 4 hours, cooled to 0 °C, filtered and washed with ether (50 ml). Recrystallisation of the crude solid from DMF-EtOH (1:1 v/v) afforded the amide **BSU-9056** (1.85 g, 99%) as a pale yellow solid; mp 240 °C dec; NMR δ(DMSO) 1.40 (4H, m, $(CH_2CH_2)_2CH_2$), 1.51 (8H, m, $N(CH_2CH_2)_2$), 2.42 (8H, m, $N(CH_2CH_2)_2$), 2.56 (4H, t, J = 5.8 $COCH_2CH_2$), 2.65 (4H, t, J = 5.8, $COCH_2$), 8.04 (2H, dd, J = 8.5, 2.0, H-3,6), 8.15 (2H, d, J = 8.5, H-4,5), 8.45 (2H, d, J = 2.0, H-1,8), 10.80 (2H, s, NH); MS (rel intensity) m/z 517 (100), 329 (12), 307 (43), 289 (25), 259 (11); Calcd ($[M+1]^+$) 517.2815. Found 517.2840; Anal. Calcd ($C_{30}H_{36}N_4O_4.0.5H_2O$): C, 68.55; H, 7.09; N, 10.66. Found

C, 68.49; H, 6.92; N, 10.66.

Example 23

2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione maleate salt BSU-9057.

General Procedure

A solution of amino amide **BSU-9056** (0.516 g, 1 mmol) in acetone (100 ml) was added a solution of maleic acid (0.116 g, 1 mmol) in MeOH (4 ml) and the solution stirred at room temperature for 30 minutes. The resulting mixture was reduced in volume and ether (25 ml) was added slowly. The resulting precipitate was filtered, washed with dry ether and dried *in vacuo* at 25 °C to give the maleate **BSU-9057** (0.58 g, 92%) as a yellow solid. mp 136-140 °C.

Example 24

2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide BSU-9058.

General Procedure

A mixture of amino amide **BSU-9056** (0.516 g, 1 mmol) and iodomethane (3.3 ml, 50 mmol) in acetone (100 ml) was stirred at room temperature for 24 h. The resulting mixture was reduced in volume, filtered, washed with dry ether and dried *in vacuo* at 25 °C to give dimethiodide **BSU-9058** (0.76 g, 95%) as a yellow solid. mp 155 °C dec.

Example 25

2,7-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione BSU-9059.

Chloroamide **BSU-3304** was treated with pyrrolidine according to the general aminolysis procedure to give amide **BSU-9059** (1.68 g, 95%) as a pale yellow solid; mp 232 °C dec; NMR δ(DMSO) 1.69 (8H, m, $N(CH_2CH_2)_2$), 2.49 (8H, m, $N(CH_2CH_2)_2$), 2.56 (4H, t, J = 6.5, $COCH_2CH_2$), 2.76 (4H, t, J = 6.5, $COCH_2$), 8.04 (2H, d, J = 8.5, H-3,6), 8.15 (2H,

d, J = 8.5, H-4,5), 8.45 (2H, s, H-1,8), 10.65 (2H, s, NH); MS (rel intensity) m/z 489 (100), 307 (20), 289 (12); Calcd ([M+1]$^+$) 489.2502. Found 489.2520; Anal. Calcd ($C_{28}H_{32}N_4O_4.0.5H_2O$): C, 67.59; H, 6.68; N, 11.26. Found C, 67.60; H, 6.46; N, 11.27. Maleate salt (**BSU-9060**), mp 172-174 °C dec. Dimethiodide, (**BSU-9061**), mp 216-218 °C dec.

Example 26

2,7-Bis(3-morpholinopropionamido)anthracene-9,10-dione BSU-9062.

Chloroamide **BSU-3304** was treated with morpholine according to the general aminolysis procedure except the mixture was heated at reflux for 5 hours to give amide **BSU-9062** (1.86 g, 99%) as a pale yellow solid; mp 235 °C dec; NMR δ(DMSO) 2.43 (8H, m, N(CH_2CH_2)$_2$O), 2.58-2.67 (8H, m, COCH_2CH_2), 3.58 (8H, m, N(CH_2CH_2)$_2$O), 8.05 (2H, dd, J = 8.5, 2.1, H-3,6), 8.15 (2H, d, J = 8.5, H-4,5), 8.47 (2H, d, J = 2.1, H-1,8), 10.71 (2H, s, NH); MS (rel intensity) m/z 521 (100), 329 (19), 307 (73), 289 (42); Calcd ([M+1]$^+$) 521.2400. Found 521.2420; Anal. Calcd ($C_{28}H_{32}N_4O_6.0.75H_2O$): C, 62.97; H, 6.32; N, 10.49. Found: C, 63.02; H, 5.89; N, 10.32. Maleate salt (**BSU-9063**), mp 130-135 °C dec. Dimethiodide, (**BSU-9064**), mp 238 °C dec.

Example 27

2,7-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione BSU-9065.

Chloroamide **BSU-3304** (1.50 g, 3.6 mmol) was treated with dimethylamine (10 ml of a 5.6M solution in EtOH) according to the general aminolysis procedure to give amide **BSU-9065** (1.48 g, 94%) as a pale yellow solid; mp 202-203 °C; NMR δ(DMSO) 2.18 (12H, s, CH_3), 2.55 (8H, m, COCH_2CH_2), 8.05 (2H, d, J = 9.0, H-3,6), 8.15 (2H, d, J = 9.0, H-4,5), 8.46 (2H, s, H-1,8), 10.68 (2H, s, NH); MS

(rel intensity) *m/z* 437 (100), 329 (11), 307 (42), 289 (22); Calcd ([M+1]$^+$) 437.2189. Found 437.2170; Anal. Calcd ($C_{24}H_{28}N_4O_4.1.25H_2O$): C 62.8; H 6.7; N 12.21. Found C 62.76; H 6.53; N 12.06. Maleate salt (**BSU-9066**), mp 167-169 °C dec. Dimethiodide, (**BSU-9067**), mp 223-224 °C, Anal. Calcd ($C_{26}H_{34}N_4O_4I_2.0.75H_2O$): C 42.55; H 4.88; N 7.63; I 34.58. Found C 42.64; H 4.88; N 7.54; I 33.29.

Example 28

2,7-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione BSU-9068.

Chloroamide **BSU-3304** was treated with diethylamine according to the general aminolysis procedure to give amide **BSU-9068** (1.56 g, 88%) as a pale yellow solid; mp 215 °C; NMR δ(DMSO) 0.98 (12H, t, J = 7.1, CH_3), 2.50 (12H, qt, J = 7.1, 7.0, NCH_2), 2.78 (4H, t, J = 7.0, $COCH_2$), 8.04 (2H, dd, J = 8.5, 2.1, H-3,6), 8.15 (2H, d, J = 8.5, H-4,5), 8.45 (2H, d, J = 2.1, H-1,8), 10.75 (2H, s, NH); MS (rel intensity) *m/z* 493 (100), 477 (12); Calcd ([M+1]$^+$) 493.2815. Found 493.2800; Anal. Calcd ($C_{28}H_{36}N_4O_4.0.25H_2O$): C, 67.65; H, 7.4; N, 11.27. Found: C, 67.64; H, 7.21; N, 11.20. Maleate salt (**BSU-9069**), mp 154-156 °C; Dimethiodide, (**BSU-9070**), mp 196 °C.

Section B - Biological Assay

Biological assays are performed as follows:

An "in vitro" Telomeric repeat amplification protocol" TRAP assay using a standard telomerase protein extract from A2780 human ovarian carcinoma cells was carried out. In previous experiments, A2780 and A2780cisR cells, where the latter represent a derived cisplatin-resistant strain, have been shown to exhibit telomerase activity.

"in vitro" TRAP assay.

A modified TRAP assay (Mieczyslaw *et al*, Methods in Cell Science, 17: 1-15, 1995) is used involving quantitative PCR and harvesting of radiolabelled telomeric TTAGGG repeats on filters and quantification by liquid scintillation counting.

A2780 cells are lysed in a CHAPS lysis buffer which comprises 0.5% CHAPS (3-[(3-cholamidopropyl)-dimethylammino]-1-propanesulfonate), 10mM Tris-HCl [pH 7.5], 1mM $MgCl_2$, 1mM EGTA, 5mM β mercaptoethanol, 10% glycerol, 0.1mM AEBSF [freshly added]). 0.04 μg of protein extract from A2780 cells in CHAPS lysis buffer is added to a PCR master mix in sterile Eppendorfs. The PCR master mix contains:

26.95μl sterile water (to give final volume of 34μl); 4μl TRAP buffer (final concentration: 20mM Tris-HCl (pH 8.3), 68mM KCl, 1.5mM $MgCl_2$, 1mM EDTA, 0.05% Tween 20); 1.25μl 2mM dNTP's; 1μl TS "forward" left primer (100μg/ml); 0.5μl BSA at 100μg/ml; and 3μCi δ-^{32}P dCTP (at 10mCi/ml = 0.3μl)
The forward primer is of the following sequence:
5'AATCCGTCGAGCAGAGTT 3'.

The following controls are run in each assay:
A. lysis buffer (2μl).
B. Heat inactivation control (85° for 10 mins).
C. 2μl of "half-strength" protein extract (4μl of 125μg/ml) = 0.2μg
D. untreated protein alone (0.04μg protein) (2μl)
E. 2μl of quarter strength protein extract to check for quantitation.

4μl of a compound of the invention dissolved in water at 500μM (or water) is then added at final concentrations

of 50, 20, 10, 5 and 1μM.

These samples are then transferred to a PCR machine and held at 25°C for 20mins followed by 80°C for 5 mins. (for the taq control drug is added at final concentration of 50μM at this stage). The following "hot-start" PCR mix is then added to each tube:

7.6μl water

1μl CX reverse primer (100μg/ml)

primer = 3' AATCCCAATCCCAATCCCAATCCC 5'

1μl 10X TRAP buffer

0.4μl of 5U/μl Taq polymerase

and samples subjected to 31 PCR cycles of 94°C denaturing 30s; 50°C annealing 30s; 72°C 1 min.

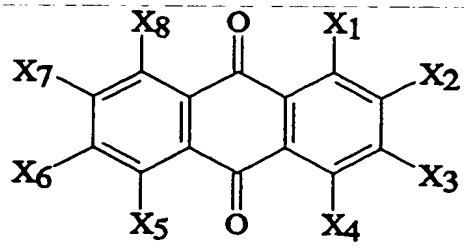
Samples are then quickly pulse vortexed and 40μl of PCR reaction transferred into a 1.5ml eppendorf tube. 800μl of 5% trichloroacetic acid (TCA) with 20mM tetrasodium pyrophosphate is added and samples left for 1hr on ice. TCA-precipitated PCR products are then harvested on Whatman filters (Millipore Unit) and filters washed with 10ml 5% TCA mix and 10ml 70% ethanol for 5 mins to dryness. The amount of radioactivity present on each filter is then determined by liquid scintillation counting. Results for each agent are expressed relative to the untreated protein alone control (minus heat inactivation control).

Table 1 below shows the assay results obtained for a selection of salt of the anthraquinones of the invention.

Salt of Anthraquinone of Example No.	BSU Number	Telomerase Inhibition (CONC)					50% INHIB (μM)
		(50μM)	(20μM)	(10μM)	(5μM)	(1μM)	
4	BSU 9011	81.9	72.3	51.6	37.3	10.7	8.6
5	BSU 9014	96.6	81.2	55.9	28.3	8.9	8.8
6	BSU 9016	19.9	10.3	8.3	0	3.9	>50
6	BSU 9017	82	58.5	40.5	-	18.6	14
7	BSU 9020	93.2	62.3	40.9	19.3	2.9	13.2
8	BSU 9023	78.2	57	29	21.4	0.6	16.8
13	BSU 9043	96.1	82.9	58.9	36.8	15.5	7.8
14	BSU 9046	97.4	88.8	53.8	36.2	1.8	8.2
16	BSU 9048	40.8	33.1	14.5	1.4	0	>50
15	BSU 9049	94.8	74.4	50.1	8.4	0	10
17	BSU 9051	100	100	80.1	37.2	37.4	6.4
17	BSU 9052	100	100	72.5	53.9	33.4	4.4
18	BSU 9054	100	91.9	82.1	55.9	6.6	4.2
18	BSU 9055	100	91	62	35.6	0	7.5
23	BSU 9057	100	100	100	79.5	0.6	3.1
24	BSU 9058	96	94.8	65.4	24.5	10.2	7.8
26	BSU 9064	82.6	64.5	23.2	12	2.3	16.5
27	BSU 9066	92.8	94.2	94.1	51.8	17.9	4.7

CLAIMS

1. An anthraquinone of formula I or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof:



(I)

in which:

each of X_1, X_4, X_5 and X_8, which are the same or different, is H, $HNCO(CH_2)_nNR^1R^2$, OH, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of X_1, X_4, X_5 and X_8 is $HNCO(CH_2)_nNR^1R^2$, and at most three of X_1, X_4, X_5 and X_8 are $HNCO(CH_2)_nNR^1R^2$ and provided that when X_1 and X_4 are both $HNCO(CH_2)_nNR^1R^2$ X_5 or X_8 is $HNCO(CH_2)_nR^1R^2$;

each of R^1 and R^2, which are the same or different, is an unsubstituted or substituted alkyl group or R^1 and R^2 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

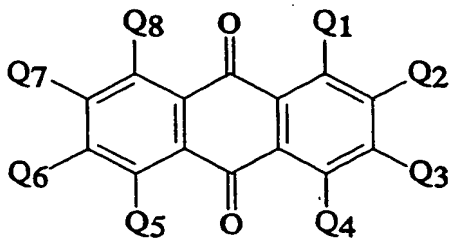
each of X_2, X_3, X_6 and X_7, which are the same or different, is H, an unsubstituted or substituted alkyl group or halogen;

provided that:

when X_1 is $HNCO(CH_2)_nNR^1R^2$, each of X_2 to X_8 is hydrogen and n is 2, either R^1 and R^2 do not both represent ethyl, or R^1 and R^2 together with the nitrogen atom to which they are attached do not represent piperidino or 2-hydroxymethyl-piperidino; or

an anthraquinone of formula II or a pharmaceutically

acceptable acid addition salt or quaternary ammonium salt thereof:



in which:

each of Q_2, Q_3, Q_6 and Q_7, which are the same or different, is H, $HNCO(CH_2)_nNR^3R^4$, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of Q_2, Q_3, Q_6 and Q_7 is $HNCO(CH_2)_nNR^3R^4$, and at most three of Q_2, Q_3, Q_6 and Q_7 are $HNCO(CH_2)_nNR^3R^4$, and provided that when Q_2 and Q_6 are both $HNCO(CH_2)_nNR^3R^4$ Q_3 or Q_7 is $HNCO(CH_2)_nR^3R^4$;

each of R^3 and R^4, which are the same or different, is an unsubstituted or substituted alkyl group or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of Q_1, Q_4, Q_5 and Q_8, which are the same or different is H, OH, an amino or substituted amino group, an unsubstituted or substituted alkyl group or halogen.

2. An anthraquinone according to claim 1 having two $HNCO(CH_2)_nNR^1R^2$ or $HNCO(CH_2)_nNR^3R^4$ groups or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof.

3. A compound according to claim 1 in which each group R^1 is the same and each group R^2 is the same or in which each group R^3 is the same and each group R^4 is the same.

4. A compound according to claim 1 wherein n is 2.

5. A compound according to claim 1 wherein R^1 and R^2 or R^3 and R^4 are the same.

6. A compound according to claim 1 wherein R^1 and R^2 or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted pyrrolidino, morpholino or piperidino group.

7. A compound according to claim 1 wherein X_1 and X_5 are $HNCO(CH_2)_nNR^1R^2$.

8. A compound according to claim 7, wherein X_2, X_3, X_4, X_6, X_7 and X_8 are each H.

9. A compound according to claim 1 wherein X_1 and X_8 are $HNCO(CH_2)_nNR^1R^2$.

10. A compound according to claim 9 wherein X_2, X_3, X_4, X_5, X_6 and X_7 are each H.

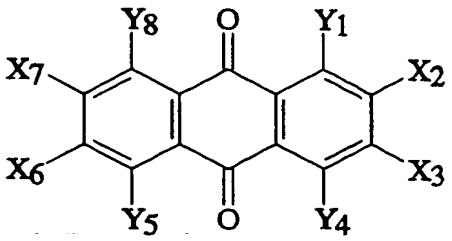
11. A compound according to claim 1 wherein Q_2 and Q_7 are $HNCO(CH_2)_nNR^3R^4$.

12. A compound according to claim 11 wherein Q_1, Q_3, Q_4, Q_5, Q_6 and Q_8 are each H.

13. A compound according to claim 1 selected from:
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
1,5-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
1,5-Bis(3-morpholinopropionamido)anthracene-9,10-dione;
1,5-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;1,5-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione;
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt;
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
1,8-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione;
1,8-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
1,8-Bis[3-(diethylamino)propionamido]anthracene-9,10-

dione;
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt;
1,5-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione diacetate salt;
1,8-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide;
1,8-Bis(3-morpholinopropionamido)anthracene-9,10-dione maleate salt;
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione;
2,7-Bis(3-pyrrolidinopropionamido)anthracene-9,10-dione;
2,7-Bis(3-morpholinopropionamido)anthracene-9,10-dione;
2,7-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
2,7-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione;
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione maleate salt; and
2,7-Bis(3-piperidinopropionamido)anthracene-9,10-dione *N,N'*-Dimethiodide.

14. A process for the production of an anthraquinone according to claim 1, which process comprises:
i) reacting a intermediate of formula (B):



(B)

in which:

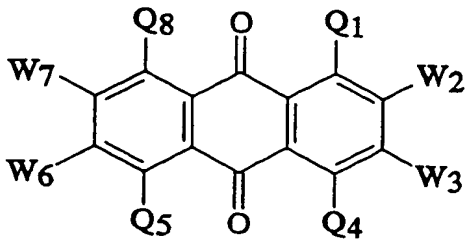
each of Y_1, Y_4, Y_5 and Y_8, which are the same or different, is H, $HNCO(CH_2)_nZ$, OH, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of Y_1, Y_4, Y_5 and Y_8 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_6 and X_7 as defined in claim 1;

with the compound of formula (C):

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined in claim 1; or

ii) reacting a intermediate of formula (A):



(A)

in which:

each of W_2, W_3, W_5 and W_7, which are the same or different, is H, $HNCO(CH_2)_nZ$, an unsubstituted or substituted alkyl group, an amino or substituted amino group or halogen, provided that at least one of W_2, W_3, W_6 and W_7 is $HNCO(CH_2)_nZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_4, Q_5 and Q_8 are as defined in claim 1;

with a compound of formula (D):

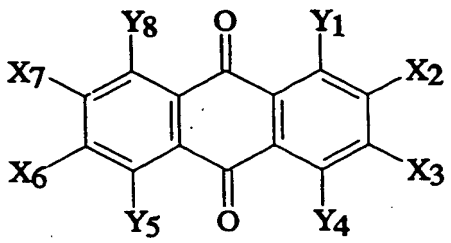
$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 as defined in claim 1.

15. A process for producing an anthraquinone of formula (I) as defined in claim 1 in which at least two of X_1, X_4, X_5 and X_8 are $HNCO(CH_2)_nR^1R^2$ and in which at least two of the groups R^1 are not the same and/or at least two of

the groups R^2 are not the same, which process comprises:-

(i) reacting an intermediate of formula (B')



(B')

in which:

each of Y_1, Y_4, Y_5 and Y_8, which are the same or different is, H, $HNCO(CH_2)_nZ$, OH, an unsubstituted or substituted alkyl group, an amino or substituted amino group, halogen or NO_2, provided that at least one of Y_1, Y_4, Y_5 and Y_8 is $HNCO(CH_2)_nZ$ and at least one of Y_1, Y_4, Y_5 and Y_8 is NO_2, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_6 and X_7 are as defined in claim 1, with a compound of formula (C)

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined in claim 1 to convert the or each group $HNCO(CH_2)_nZ$ to a group X_1, X_4, X_5 or X_8 which is $HNCO(CH_2)_nNR^1R^2$ as defined in claim 1;

(ii) converting the or each group NO_2 group to an NH_2 group;

(iii) reacting the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, to convert the or each NH_2 group into $HNCO(CH_2)_nZ$;

(iv) reacting the product of step (iii) with a compound of formula (C'):

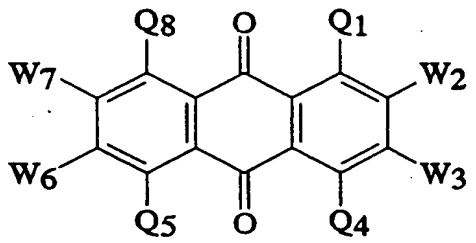
$$R^{1'}R^{2'}NH \qquad (C)$$

wherein $R^{1'}$ and $R^{2'}$ have the same definition as R^1 and

R^2 in claim 1, with the proviso that the compound of formula (C') is not identical to the compound of formula (C) used in step (i), to give a compound of formula (I); or

a process for producing an anthraquinone of formula (II) as defined in claim 1 in which at least two of Q_2, Q_3, Q_6 and Q_7 are $HNCO(CH_2)_nR^3R^4$ and in which at least two of the groups R^3 are not the same and/or at least two of the groups R^4 are not the same, which process comprises:

(i) reacting an intermediate of formula (A'):



(A')

in which:

each of W_2, W_3, W_6 and W_7, which are the same or different is, H, $HNCO(CH_2)_nZ$, an unsubstituted or substituted alkyl group, an amino or substituted amino group, halogen or NO_2, provided that at least one of W_2, W_3, W_6 and W_7 is $HNCO(CH_2)_nZ$ and at least one of W_2, W_3, W_6 and W_7 is NO_2, wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_4, Q_5 and Q_8 are as defined in claim 1; with a compound of formula (D):

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 are as defined in claim 1, to convert the or each group $HNCO(CH_2)_nZ$ to a group Q_2, Q_3, Q_6 or Q_7 which is $HNCO(CH_2)_nNR^3R^4$ as defined in claim 1;

(ii) converting the or each group NO_2 group to an NH_2 group;

(iii) reacting the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer

of from 1 to 6, to convert the or each NH_2 group into $HNCO(CH_2)_nZ$;

(iv) reacting the product of step (iii) with a compound of formula (D'):

$$R^{3'}R^{4'}NH \qquad (D')$$

wherein $R^{3'}$ and $R^{4'}$ have the same definition as R^3 and R^4 in claim 1, with the proviso that the compound of formula (D') is not identical to the compound of formula (D) used in step (i), to give a compound of formula (I).

16. A process for the production of a quaternary ammonium salt of an anthraquinone of formula I or formula II according to claim 1 which process comprises treating an anthraquinone of formula I or II with an alkylating agent.

17. A compound according to claim 1 for use in the inhibition of telomerase.

18. A compound according to claim 17 for use in the treatment of cancer.

19. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier or diluent thereof.

20. Use of a compound according to claim 1 in the manufacture of a medicament for inhibiting the activity of telomerase.

21. Use according to claim 20 for the manufacture of a medicament for use in the treatment of cancer.

22. A method of treating a host suffering from cancer which method comprises administering thereto a pharmaceutical effective amount of a compound of formula (I) or formula (II) as defined in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/03444

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C237/04 A61K31/16 C07D295/14 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 00265 A (CANCER RESEARCH TECHNOLOGY LTD) 10 January 1991 cited in the application see claims; examples	1-22
A	AGBANDJE, MAVIS ET AL: "Anthracene-9,10-diones as potential anticancer agents. Synthesis, DNA-binding, and biological studies on a series of 2,6-disubstituted derivatives" J. MED. CHEM. (1992), 35(8), 1418-29 CODEN: JMCMAR;ISSN: 0022-2623, XP002063825 cited in the application see page 1419	1-22

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 1998	25/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sánchez García, J.M.

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 97/03444

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TANIOUS, FARIAL A. ET AL: "Substituent position dictates the intercalative DNA-binding mode for anthracene-9,10-dione antitumor drugs" BIOCHEMISTRY (1992), 31(46), 11632-40 CODEN: BICHAW;ISSN: 0006-2960, XP002063826 cited in the application see page 11632	1-22
A	COLLIER, DAVID A. ET AL: "Synthesis, molecular modeling, DNA binding, and antitumor properties of some substituted amidoanthraquinones" J. MED. CHEM. (1988), 31(4), 847-57 CODEN: JMCMAR;ISSN: 0022-2623, XP002063827 cited in the application see page 847 - page 848	1-22
A	US 3 859 315 A (SANTILLI ARTHUR A ET AL) 7 January 1975 see claims	1-22
A	WO 86 00892 A (BIBER RUDOLF) 13 February 1986 see claims	1-22
A	HOFFMANN, SIEGFRIED ET AL: "Mono- and bis-basic anthraquinones" Z. CHEM. (1986), 26(6), 206-7 CODEN: ZECEAL;ISSN: 0044-2402, XP002063828 see page 206	1-22
A	WINKELMANN, E. ET AL: "Chemotherapeutically active anthraquinones. I. Aminoanthraquinones" ARZNEIM.-FORSCH. (1979), 29(10), 1504-9 CODEN: ARZNAD;ISSN: 0004-4172, XP002063829 see page 1505 - page 1507	1-22
A	MARTELLI, SANTE ET AL: "Synthesis and antineoplastic evaluations of 1,4-bis(aminoalkanamido)-9,10-anthracenediones" J. MED. CHEM. (1988), 31(10), 1956-9 CODEN: JMCMAR;ISSN: 0022-2623, XP002063830 see page 1956 - page 1957	1-22
A	GATTO, BARBARA ET AL: "Peptidyl Anthraquinones as Potential Antineoplastic Drugs: Synthesis, DNA Binding, Redox Cycling, and Biological Activity" J. MED. CHEM. (1996), 39(16), 3114-3122 CODEN: JMCMAR;ISSN: 0022-2623, XP002063831 see page 3115	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/GB 97/03444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9100265 A	10-01-1991	EP 0482119 A	29-04-1992
US 3859315 A	07-01-1975	NONE	
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		EP 0191058 A	20-08-1986
		JP 61502891 T	11-12-1986
		US 4794125 A	27-12-1988